DECLARATION

I, the undersigned, Izumi UCHIYAMA of c/o ARCO PATENT OFFICE, Bo-eki Bldg., 123 Higashi-machi, Chuo-ku, Kobe-shi, Hyogo 650-0031 JAPAN hereby declare that I am conversant with Japanese and English languages and that attached is, to the best of my knowledge and belief, a true and accurate English translation of the Japanese specification and claims of the U.S. Patent Application No.10/674,787 filed on October 1, 2003 entitled "Method for Detecting Extension Reaction with Primers, Method for Distinguishing Kind of Bases, Apparatus for Distinguishing Kind of Bases, Apparatus for Detecting Pyrophosphate, Method for Detecting Nucleic Acid, and Tip to Mount Sample Solution"

So declared in Kobe, Japan,
This 19th day of January, 2004

内山泉

Izumi UCHIYAMA

AMENDMENTS TO THE SPECIFICATION

Please amend the first full paragraph on page 10 as follows:

In the second method, pyrophosphate is brought into the action of glycerol-3-phosphate cytidyl transferase in the presence of cytidine diphosphate ~~diphosphorus~~ glycerol. Glycerol triphosphate is produced by this reaction. Therefore, the amount of pyrophosphate can be derived by calculation through measuring the amount of thus produced glycerol triphosphate. As methods of measuring the amount of glycerol triphosphate, two kinds of methods have been proposed. One is a method in which increase of NAD(P)H is colorimetrically determined upon oxidation of glycerol triphosphate with NAD(P) utilizing a catalytic action of glycerol-3-phosphate dehydrogenase. In another method, hydrogen peroxide, which is produced by bringing thus produced[[b]] glycerol triphosphate into the action of glycerol-3-phosphate oxidase, is introduced to a pigment to allow for a colorimetric determination. Another is a method in which colorimetric determination is carried out through introducing hydrogen peroxide, which is produced by bringing thus produced, to a pigment.

Please amend the second paragraph on page 17 as follows:

In the step (d), the concentration of H^+ may be optically measured. In this instance, for example, the H^+ concentration can be measured by adding a pH sensitive pigment or a membrane potential sensitive pigment to at least either one of the solution at the aforementioned front face side and the solution at the back face side, and measuring an optical response of the aforementioned pigment. Exemplary pH sensitive pigment described above includes e.g.,

acridine orange. Exemplary membrane potential sensitive pigment described above includes e.g., Oxonol ~~Oxol~~ V.

Please amend the first full paragraph on page 22 as follows:

In the step (d), the H^+ concentration of may be optically measured. In this instance, the H^+ concentration can be measured by, for example, adding a pH sensitive pigment or a membrane potential sensitive pigment to at least either one of the solution at the front face side and the solution at the back face side, and measuring an optical response of the aforementioned pigment. Exemplary pH sensitive pigment described above includes e.g., acridine orange. Exemplary membrane potential sensitive pigment described above includes e.g., Oxonol ~~Oxol~~ V.

Please amend the fifth paragraph on page 24 as follows:

Fig. 7 (a) is a top view schematically showing the tip according to Embodiment 1; and Fig. 7 (b) is a cross sectional view along the line X-X depicted in Fig. 7(a).

Please amend the second full paragraph on page 27 as follows:

Next in the step illustrated in Fig. 1 (c), the sample solution is cooled to allow hybridization of the single stranded DNA 4 to the typing primer 7. Because the SNP site S1 of the single stranded DNA 4 is adenine (hereinafter, denoted as A), the typing primer 7 completely hybridizes to the single stranded DNA 4. Similarly, in the step illustrated in Fig. 2 (c), the sample solution is cooled to allow hybridization of the single stranded DNA 6 to the typing primer 7. Because the SNP site S2 of the single stranded DNA 6 is guanine (hereinafter, denoted as G), the typing primer 7 does not hybridize to the single stranded DNA 6 [[4]] only at its 3' end base (T).

Please amend the first full paragraph on page 35 as follows:

Exemplary pH sensitive pigment includes acridine orange. Further, exemplary membrane potential sensitive pigment includes Oxonol ~~Oxol~~ V. Both of these are extremely sensitive pigments on a slight change of pH or membrane potential. Accordingly, detection of pyrophosphate with high sensitivity is enabled.

Please amend the second full paragraph on page 43 as follows:

Next, another tip 53a which can be used instead of the tip 53 is explained. Fig. 7 (a) is a top view schematically showing another tip according to this Embodiment, and Fig. 7 (b) is a cross sectional view along the line X-X depicted in Fig. 7(a).

Please amend the third full paragraph on page 52 as follows:

(Detection Experiment of pyrophosphate 1)

This Experiment ~~Example~~ was conducted according to the method of Shizuo Yoshida et al., (Masayoshi Maeshima and Shizuo Yoshida, 1989, J.Biol.Chem., 264(33), pp. 20068-20073) as demonstrated below.

Please amend the second full paragraph on page 53 as follows:

Next, this H^+-pyrophosphatase liquid was evenly dispensed into 6 [[4]] tubes, and thereto was added a sodium pyrophosphate solution such that each final concentration of sodium pyrophosphate became 10 μM, 20 μM, 40 μM, 60 μM, 80 μM and 100μM, respectively, to initiate the hydrolysis reaction of pyrophosphate by H^+-pyrophosphatase.

Please amend the second paragraph on page 54 as follows:

(Detection Experiment of pyrophosphate 2)

This Experiment ~~Example~~ was conducted according to the method of Masasuke Yoshida et al., (MasaH.Sato, Masahiko Kasahara, Noriyuki Ishii, Haruo Homareda, Hideo Matsui and Masasuke Yoshida, 1994, J.Biol.Chem., 269(9), pp. 6725-6728) as demonstrated below.

Please amend the last paragraph on page 54 as follows:

Subsequently, to the 6 [[5]] tubes was added a sodium pyrophosphate solution such that final concentration of sodium pyrophosphate became 10 μM, 20 μM, 40 μM, 60 μM, 80 μM and 100μM, respectively, to initiate the hydrolysis reaction of pyrophosphate by H^+-pyrophosphatase.

Please amend third full paragraph on page 55 as follows:

(Detection Experiment of pyrophosphate 3)

This Experiment ~~Example~~ was conducted according to the method disclosed in JP-A No. 6-90736.

Please amend the last paragraph on page 55 and continuing onto page 56 as follows:

First, similarly to Detection Experiment of pyrophosphate 2 ~~Example 2~~ described above, a lipid bilayer including tonoplast membrane H^+-pyrophosphatase was fixed on a commercially available ISFET-pH sensor using tonoplast membrane H^+-pyrophosphatase derived from seeds of squash. It should be noted however that outside of the lipid bilayer was filled with a reaction solution containing $MgSO_4$ (concentration of 1 mM), KCl (concentration of 50 mM), sorbitol (concentration of 0.25 M), Hepes/Bristris propane (concentration of 25 mM, pH 7.2).

Please amend the fourth paragraph on page 56 as follows:

First, a sample liquid A containing λDNA (manufactured by Takara Shuzo Co., Ltd.) at the concentration of 10 ng/μL dissolved in distilled water, and a sample liquid B consisting of distilled water alone were provided. Also, as shown in Fig. 14 (a), primer solutions E and F containing two kinds of primer[s] C (SEQ ID NO:1) and primer D (SEQ ID NO:2), which can completely hybridize to a particular base sequence of λDNA, dissolved in distilled water (20 μM each), respectively, were provided.

Please amend the second full paragraph on page 57 as follows:

After terminating the PCR reaction, each of the PCR reaction liquids G and H was mixed with the H^+-pyrophosphatase liquid described in the above Detection Experiment of pyrophosphate 1 ~~Example 1~~ to subject to a reaction.

Please amend the fourth full paragraph on page 58 as follows:

Next, in order to discriminate the difference of the bases described above, a typing primer (SEQ ID NO:3) illustrated in Fig. 16 (a) was provided. Subsequently, a typing primer solution was prepared by dissolving the typing primer in distilled water to give the final concentration of 20 μM.

Please amend the last paragraph on page 58 as follows:

The typing primer illustrated in Fig. 16 (a) completely hybridizes to the single stranded DNA described in the lower panel of wild type λDNA (SEQ ID NO:4). However, the base G at 3' end of this typing primer can not hybridize to the single stranded DNA described in the lower

panel of mutant λDNA (SEQ ID NO:5). Therefore, when the primer extension reaction is executed using this typing primer, the reaction satisfactorily proceeds in the instance of wild type λDNA, however, the reaction does not proceed well in the instance of mutant λDNA.

Please amend the first paragraph on page 59 as follows:

Also, the primer solution F used in the aforementioned Example 1 [[4]] was provided.

Please amend the last paragraph on page 60 and continuing onto page 61 as follows:

(Example 3)

In this Example, unlike the Example 2 [[5]] described above, possible discrimination of the difference of a single base pair between wild type λDNA and mutant λDNA was studied with a method of combination of a 1 base extension reaction and a reaction of H^+-pyrophosphatase.

Please amend the second paragraph on page 61 as follows:

Next, the primer (SEQ ID NO:6) illustrated in Fig. 18 (a) was provided. This primer can completely hybridize to the single stranded DNA shown in the lower panel of wild type λDNA which is illustrated in Fig. 16 (a) in Example 2 [[5]], at the sequence other than the base C at its 5' end. In other words, similarly to the single stranded DNA sequence shown in the lower panel of the mutant λDNA ssequence demonstrated in Example 2 [[5]], it can completely hybridize to the sequence other than the base T at its 5' end.

Please amend the last paragraph on page 61 and continuing onto page 62 as follows:

After terminating the single base extension reaction, each extension reaction liquid was introduced to a modified ISFET electrode including H^+-pyrophosphatase fixed thereto. The modified ISFET electrode was that used in the aforementioned Detection Experiment of pyrophosphate 3 ~~Example 3~~.